Pergamon

Tetrahedron Letters, Vol. 38, No. 11, pp. 1971-1974, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00251-7

A Variety of Lipid-coated Glycoside Hydrolases as Effective Glycosyl Transfer Catalysts in Homogeneous Organic Solvents

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Abstract: Various lipid-coated glycoside hydrolases were prepared, which act as efficient catalysts for transglycosylation of α-D- and β-D-mannoside, N-acetyl-β-D-glucosaminide, β-D-glucoside, and β-D-galactoside to hydrophobic acceptor alcohols in dry isopropyl ether. The enzyme activity for the glycosylation depended on coating lipids as well as origin or kind of enzymes.

With recent rapid advance in glycobiology, necessity of studies on effective and facile syntheses of glycoside derivatives rises. In particular, enzymatic syntheses have advantages to provide regio- and stereo-selective products without using protection groups in one step reaction, comparison with the chemical syntheses.[1] Many attempts have been performed to use glycoside hydrolases as a transglycosylation catalyst in aqueous solutions containing water-miscible organic solvents by utilizing a reverse hydrolysis reaction.[2] In these reactions, however, it has been difficult to obtain transglycosylation products in good yield, because the hydrolysis reaction proceeded fast relative to the transglycosylation due to the presence of aqueous solution. If the reaction can be carried out in non-aqueous organic solvents without denaturation of enzymes, the transglycosylation products could be obtained in a high yield.

We have recently reported a lipid-coated enzyme system, in which hydrophilic head groups of lipids interact with the enzyme surface and lipophilic two long alkyl chains extend away from its surface to solubilize the enzyme in hydrophobic organic solvents. The lipid-coated lipase,[3a-c] phospholipase,[3d] and catalytic antibody[3e] showed high catalytic activities such as enantioselective esterifications in isooctane or benzene.

In this paper, we apply our lipid-coating technique to transglycosylation by using the reverse hydrolytic activity of various glycosidases. Lipid-coated glycoside hydrolases act as efficient catalysts for transglycosylation of Man-α-, Man-β-, GlcNAc-β-, Glc-β-, and Gal-β- from the respective *p*-nitrophenyl-D-pyranosides to 5-phenyl-1-pentanol in dry isopropyl ether (Figure 1).

All lipid-coated glycoside hydrolases were prepared similarly according to our previous works.[3,4] An aqueous buffer solution (10 mL, 10 mM phosphate, pH 5.1) of the glycoside hydrolase was mixed with an aqueous dispersion (10 mL) of synthetic lipids (10 mg, shown in Figure 1) and stirred for one day. The glycoside hydrolases employed in this study were as follows and were used without further purification: β-*D*-Galactosidase from *Escherichia coli* (TOYOBO Co.), α-*D*-mannosidase from *Jack beans* (Sigma Chemicals Co.), β-*N*-acetyl-*D*-glucosaminidase from *Bovine kidney* (Sigma Chemicals Co.), β-*D*-glucosidase from *Aspergillus niger* (Novo Nordisk Co.), and β-*D*-glycosidase I from *Pyrococcus furiosus* (Takara Co.). The precipitates were gathered by centrifugation, washed with buffer solution and distilled water repeatedly, and

then lyophilized. The resulting white powders were soluble in most organic solvents such as chloroform, acetonitrile, benzene and isopropyl ether, but insoluble in aqueous buffer solution. The protein contents of the lipid-enzyme complexes were determined from both the elemental analyses (C, H, and N) and the UV absorption by aromatic amino acid residues of proteins at 280 nm in chloroform solution.[3,4] The protein contents of the lipid-enzyme complex were 6-9 wt%.

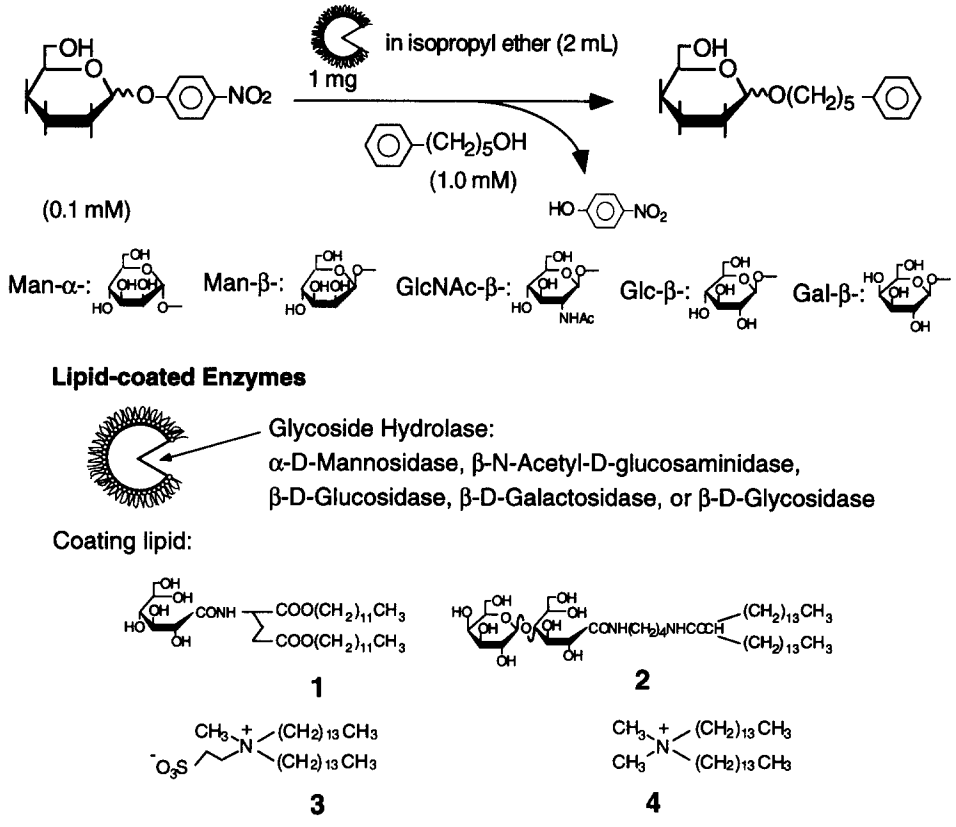

Fig. 1 Schematic illustrations of transglycosylations catalysed by lipid-coated glycoside hydrolases in isopropyl ether

Figure 2 shows typical time courses of transmannosylation from *p*-nitrophenyl-α-*D*-mannopyranoside (Man-α-pNP) as a mannosyl donor to the 10-fold excess of 5-phenyl-1-pentanol (PhC_5-OH) as a mannosyl acceptor in isopropyl ether at 30 °C. Reactions were followed by reversed phase liquid chromatography of the reduction of Man-α-pNP and productions of mannosides (Man-α-OC_5Ph) and *p*-nitrophenol that is by-product. When a native α-*D*-mannosidase was employed in acetonitrile-water (7:3) according to the conventional methods,[1] the transglycosylation product (Man-α-OC_5Ph) was obtained in very low yield (*ca.* 10 %) in 5 h and disappeared completely after 20 h, that is the mannosyl donor was completely hydrolysed to mannose and *p*-nitrophenol after 2 days (Figure 2a). The similar results have been reported in some aqueous-buffer systems catalysed by a native enzyme.[4] On the other hand, in the case of an α-*D*-Mannosidase coated with the lipid(**2**) in dry isopropyl ether, only the transglycosylated Man-α-OC_5Ph was obtained in 68 % yield

in one day, and the yield corresponded to the amount of consumed Man-α-pNP (Figure 2b). It was confirmed from ^{1}H- and ^{13}C-NMR of the isolated products that the chemical structure of Man-OC_5Ph was kept in α-configuration of *D*-mannose. When a native α–*D*-mannosidase was employed as dispersion in isopropyl ether, the transmannosylation or even hydrolysis hardly occurred probably because of denaturation or low solubility of enzymes in organic media (date not shown).

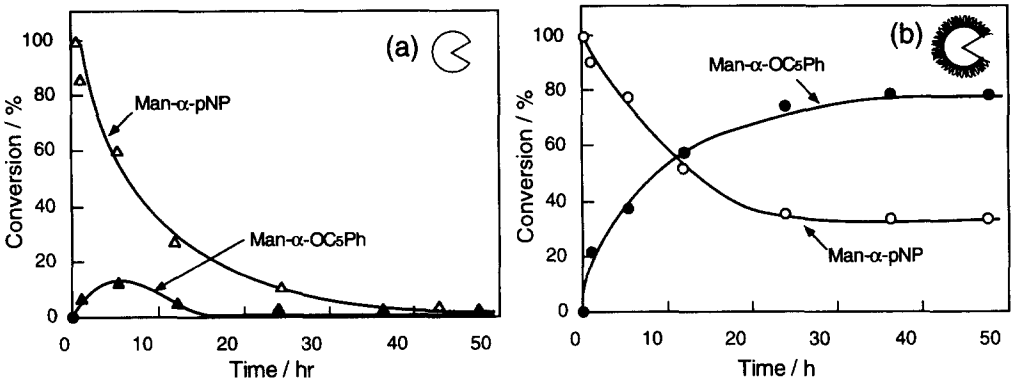

Fig. 2 Typical time courses of transmannosylation from *p*-nitrophenyl-α-D-mannopyranoside (Man-α-pNP, 0.1 mM) to 5-phenyl-1-pentanol (PhC_5OH, 1.0 mM) at 30 °C. (a) In 10 mM phosphate buffer solution (pH 5.1)-acetonitrile (3:7) catalysed by a native α-D-mannosidase, and (b) in dry isopropyl ether catalysed by a lipid(**2**)-coated α-D-mannosidase. The concentration of the enzyme is 0.1 mg of protein in 2 mL.

Table 1 Transglycosylation yields catalysed by lipid-coated glycoside hydrolases in dry isopropyl ether[a]

		Coating lipids			
Enzymes	Glycosyl donors	Nonionic (**1**)	Nonionic (**2**)	Zwitterionic (**3**)	Cationic (**4**)
α-D-Mannosidase	Man-α-pNP	60	68	0	3
β-N-Acetyl-D-glucosaminidase	GlcNAc-β-pNP	28	33	0	3
β-D-Glucosidase	Glc-β-pNP	0	23	5	10
β-D-Galactosidase	Gal-β-pNP	62	0	8	8
β-D-Glycosidase I	Glc-β-pNP	0	0	29	15
	Gal-β-pNP	0	0	71	3
	Man-β-pNP	0	0	45	3

[a]Transglycosylation from *p*-nitrophenyl-glycopyranoside (0.1 mM) to 5-phenyl-1-pentanol (1.0 mM) in dry isopropyl ether (2 mL) catalysed by the respective lipid-coated enzyme ([Enzyme] = 0.1 mg of protein) at 30 °C. Yields contain ±5% experimental errors in each three experiment.

Various lipid-coated enzymes were prepared from α–*D*-mannosidase, β-N-acetyl-D-glucosaminidase, β-D-glucosidase, β-D-galactosidase, and β-D-glycosidase I with four kinds of dialkyl amphiphiles having nonionic gluconoamide (**1**), nonionic lactonoamide (**2**), zwitterionic (**3**) and cationic (**4**) head groups. Chemical structures of lipid molecules (**1**-**4**) are shown in Figure 1. Transglycosylation yields catalysed by lipid-coated glycoside hydrolases in dry isopropyl ether are summarized in Table 1. When α-D-mannosidase from *Jack beans* was coated with sugar lipids (**1** or **2**), transglycosylation products (Man-α-C_5Ph) could be

obtained in high yields (60-68%). The catalytic activity of the enzyme coated with the zwitterionic (**3**) or the cationic (**4**) lipid was very low compared to those of complexes with nonionic amphiphiles. The strong electrostatic interaction between the cationic head groups of amphiphiles and the hydrophilic surface of the enzyme would cause the enzyme structure to denature. These results corresponded to the cases of the lipid-coated lipase.[3a]

In the case of a lipid-coated β-N-acetyl-D-glucosaminidase from *Bovine kidney,* the similar tendency was observed although the yield of GlcNAc-β-C_5Ph was relatively low (28-33%). In the transglucosylation, β-D-glucosidase from *Aspergillus niger* coated with the nonionic (**2**) and the cationic (**4**) lipids gave relatively low yields (10-23%). When β-D-galactosidase from *Escherichia coli* was employed, the nonionic lipid (**1**) showed a good yield (62%) of trans-β-D-galactosylation. Although it is difficult to explain effects of coating lipids on enzyme activities, it should be emphasised that enzyme activities of the β-D-glucosidase coated with the lipid (**1**) bearing a gluconoamide unit at the hydrophilic head group and the β-D-galactosidase coated with the galactose lipid (**2**) were completely depressed. This is probably due to the inhibition effect of active site by head groups of coating lipids.

Though the β-D-glycosidase I from *Pyrococcus furiosus* is unity by electrophoresis analysis, it can recognize β-D-galactoside, β-D-glucoside, and β-D-mannoside moieties.[5] When β-D-glycosidase I was coated with zwitterionic lipid (**3**), β-glucose, β-galactose, and β-mannose units could be transferred in fair yields (29-71%) from Glc-β-pNP, Gal-β-pNP, and Man-β-pNP, respectively. The enzymes coated with gluconoamide (**1**) and galactose (**2**) lipids did not show any enzymatic activities due to the same reasons mentioned above.

In conclusion, a variety of the lipid-coated glycoside hydrolases, such as α– and β–*D*-mannosidase, β–*D*-galactosidase, β–*D*-glucosidase, and *N*-acetyl-β–*D*-glucosaminidase were found to be used as effective transglycosylation catalysts in dry isopropyl ether. The coating lipid acts as lipophilic part to solubilize enzymes in organic solvents and affects sometimes the enzymatic activity.

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(*Received in Japan* 4 *December* 1996; *revised* 5 *February* 1997; *accepted* 7 *February* 1997)